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Conversion of Quazepam to 2-Oxoquazepam in Alkaline Solution

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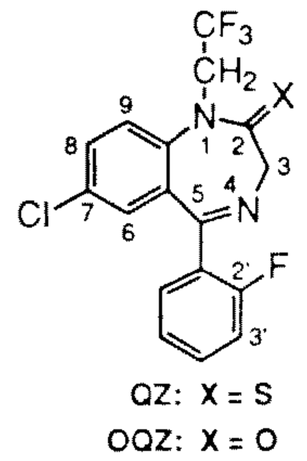
ABSTRACT

Quazepam [7-chloro-1-(N-2,2,2-trifluoroethyl)-5-(2′-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-thione, QZ], an anxiolytic drug in clinical use, was converted in an alkaline solution to form 11 detectable poroducts. 2-Oxoquazepam [7-chloro-1-(N-2,2,2-trifluoroethyl)-5-(2′-fluorophenyl)-1, 3-dihydro-2*H*-1,4-benzodiazepin-2-one, OQZ] was the most abundant product formed. Reaction kinetics in alkaline solutions were analyzed by reversed-phase high-performance liquid chromatography. Time-dependent ultraviolet-visible absorption spectral measurements indicated an initial formation of an intermediate, followed by the formation of OQZ as the most abundant product. The electron-deficient C2 carbon of QZ probably undergoes a nucleophilic addition reaction by a hydroxide ion, resulting in the formation of primarily OQZ and other minor products.

Key words : Quazepam, 2-oxoquazepam, Kinetics, Spectrophotometry

INTRODUCTION

Quazepam (QZ) and 2-oxoquazepam (OQZ) (Figure 1) are among the 1,4-benzodiazepines shown to be capable of differentiating CNS subtypes of benzodiazepine receptors.[1-9] OQZ is the major pharmacologically active metabolite of QZ.[5,10-12] OQZ exhibiting a higher potency than QZ at the CNS type I receptor sites.[6,7] The 1-*N*-trifluoroethyl substituent appears to be responsible for the receptor subtype selectivity because benzodiazepines lacking the 1-*N*-trifluoroethyl group do not discriminate among the subtypes of benzodiazepine receptors.[2] The electrophilic character of C2 carbonyl carbon induced by the electron-withdrawing properties of the 1-*N*-trifluoroethyl substituent may be related



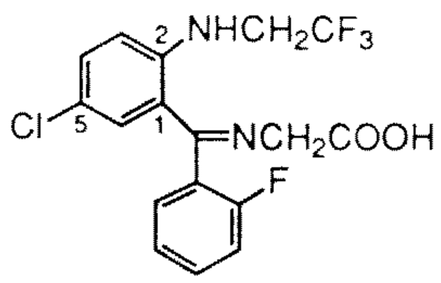


Figure 1. Strutures and abbreviations of quazepam (QZ), 2-oxoquazepam (OQZ), OQZ-HP (a hydrolysis product of OQZ), and decomposition product TFCFBP.

to the unique receptor subtype selectivity of 1-*N*-trifluoroethylated 1, 4-benzodiazepines such as halazepam, QZ, and OQZ.[13]

Conversion of QZ to OQZ occurs enzymatically both in vitro and in vivo.[10-12] Although the mechanism for the enzymatic conversion of QZ to OQZ has not been elucidated to the reaction is probably catalyzed by the cytochrome P450-containing drug metabolizing enzyme systems.

The formation of OQZ from QZ in alkaline solutions was first realized in a study by a polarographic method.[14] It was suggested that OQZ was formed by the addition of a hydroxide ion to the C2 thione carbon of QZ.[14] Further base-catalyzed hydrolysis of OQZ was not observed.[14] We have recently reported that OQZ undergoes a ring-opening hydrolysis reaction in a strongly alkaline media.[13] In this communication, we report the results of kinetic studies in hydroxide ion-induced conversion of QZ to OQZ by reversed-phase HPLC and spectrophotometry. Evidence indicated that a nucleophilic attack of the C2 carbon of the C=S double bond by a hydroxide ion initiated the conversion of QZ to OQZ. The conclusion is similar to that proposed by Oelschläger et al.,[14] who studied the same reaction by a polarographic method.

MATERIALS AND METHODS

I. *Materials*

Quazepam (QZ) and 2-oxoquazepam (OQZ) were generously provided by Schering-Plough Corporation (Bloomfield, NJ). 2-(N-2,2,2-trifluoroethylamino)-5-chloro-2′-fluorobenzophenone (TFCFBP; Figure 1) was prepared from OQZ similarly as recently described.[13] OQZ-HP was prepared by dissolving OQZ in MeCN : 0.2 M NaOH (1:1, v/v) and the solution was kept overnight at room temperature. The solution was neutralized and a hydrolysis product (OQZ-HP) was then isolated by reversed-phase HPLC.[13]

II. *HPLC*

HPLC was performed using a Waters Associates (Milford, MA) Model M45 solvent pump and a Model 441 absorbance detector (254nm). A Vydac C18 column (5μ particles, 250 mm, x 4.6 mm i.d. catalog no. 218TP54; The Separations Group, Hesperia, CA) was used. To analyze samples in kinetic experiments, MeCN : 0.02 M phosphate buffer (pH7.0) (55 : 45, 60 : 40, or 65 : 35; v/v) was used as the mobile phase at a flow rate of 1 ml/min. Samples were injected via a Shimadzu (Shimadzu Corp., Kyoto, Japan) Model SIL-9A automatic sample injector equipped with a water-jacketed sample rack. Temperature of the sample rack was maintained ($\pm$0.1°C) by passing constant-temperature water from a thermostated water circulator. Actual temperature of the solution in the sample vial was measured with a portable digital thermometer fitted with a detachable probe (Thomas Scientific, Swedesboro, NJ). The detector signal was recorded with MacIntegrator (a software and hardware package from Rainin Instruments Co., Inc., Emeryville, CA) on a Macintosh Classic II computer (Apple Computer, Cupertino, CA).

In kinetic studies, the reaction was initiated by adding 2 ml of a solvent mixture (pre-equilibrated to temperature under study) to a test tube containing appropriate amounts of dried residues of QZ (final concentration 54 or 272 μM). The mixture was then quickly vortexed for ~ 30 sec to dissolve the QZ. The resulting solution was immediately transferred to a sample vial (pre-equilibrated to the temperature under study) and placed in a sample well of the autosampler′s thermostated sample rack. The first sample was injected after the sample vial has been placed in the sample well for 5 min to allow sample's temperature to reach complete equilibrium. An Aliquot (10-μl) was subsequently injected for analysis at sampling intervals ranging from 6.4 to 15.4 min. The reproducibility of constant volume injection was within 2%.

Reaction t½ for either the disappearance

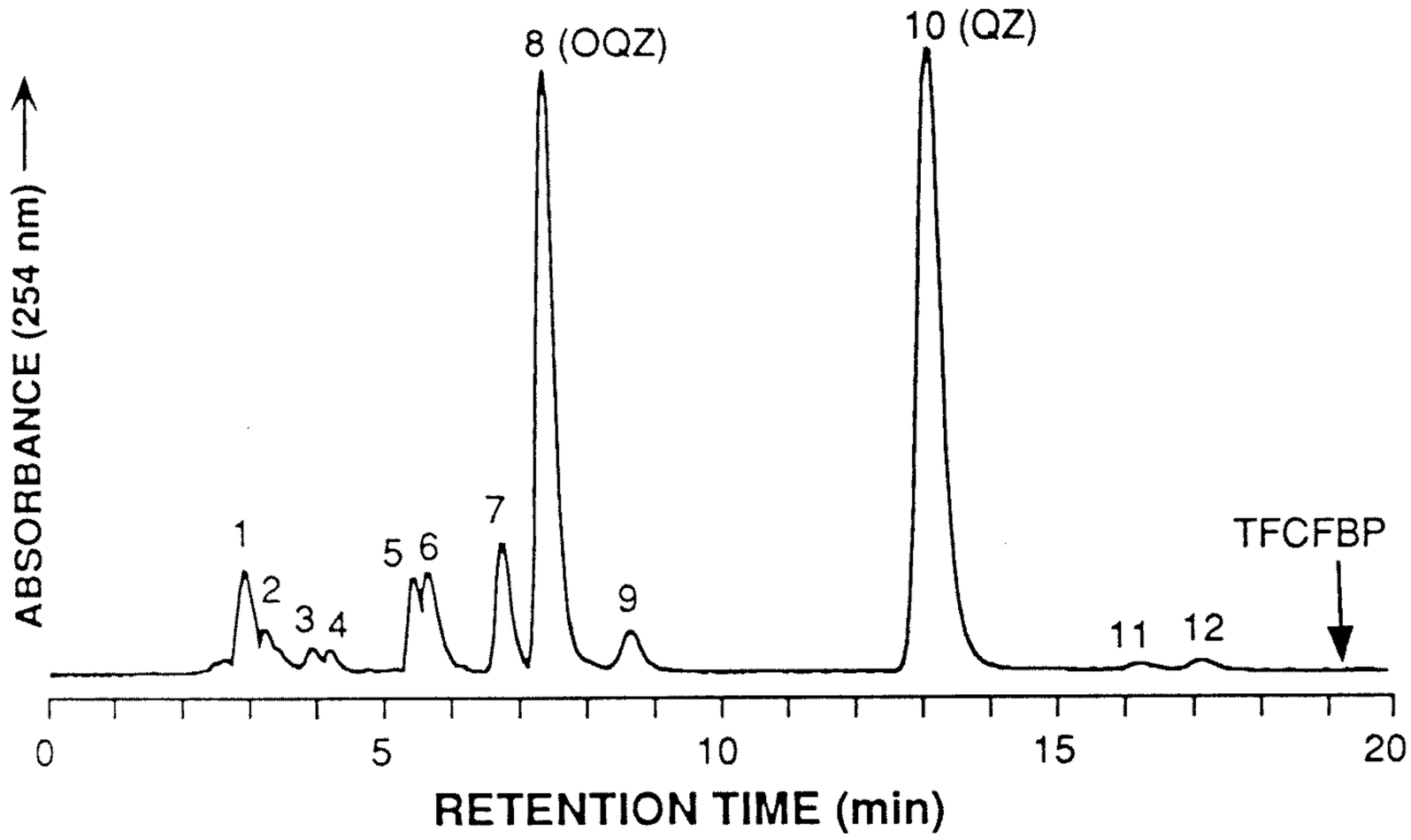


Figure 2. Reversed-phase HPLC separation of hydroxide ion-induced reaction products of QZ. The sample was derived from QZ (0.27 mM) Which has been maintained in MeCN : 2mM NaOH (1:1, v/v) at 25°C for 110 min. Peaks 2, 8 and 10 are OQZ-HP, OQZ and QZ respectively. Retention time of TFCFBP is indicated by an arrow. Chromatographic conditions are described in Materials and Methods.

of QZ or the formation of OQZ was determined by a curve fitting computer software. Reaction t½ were averages of 3 determinations.

III. *Spectral Analysis*

Uv-vis absorption spectra and kinetic analysis at a wavelength were performed using a 1 cm path length thermostated quartz cuvette on a Model DW2000 spectrophotometer (SLM Instruments, Urbana, IL). Temperature of the cuvette was maintained (±0.1°C) by passing constant-temperature water from a thermostated water circulator. Mass spectral analysis was performed on a Finnigan 4500 gas chromatograph-mass spectrometer-data system (Finnigan MAT, San Jose, CA) with a solid probe by electron impact at 70 eV and the ion source was maintained at 120°C.

RESULTS AND DISCUSSION

Product formation and kinetics by Reversed-Phase HPLC

QZ and its hydroxide ion-induced reaction products were analyzed by reversed-phase HPLC (Figure 2). The sample in Figure 2 was taken from a reaction mixture in which QZ(105μg/ml) had been in MeCN:2 mM NaOH (1:1, v/v) at 25 °C for 110 min. MeCN was used as a cosolvent to ensure solubility of QZ and its products. OQZ (peak 8) was the most abundant product formed. A minor amount of hydrolysis product OQZ-HP (peak 2) was also formed, due to further base-catalyzed hydrolysis of OQZ.[13] The possible decomposition product TFCFBP was not detectable. The product contained in peak 8 was identical to the authentic OQZ in chromatographic retention time, ultraviolet absorption and mass spectra. Under the same experimental condition, hydrolysis of OQZ had t½ > 300 min.[13]

Under the experimental conditions described in Figure 2, the kinetics in the disappearance of QZ and the formation of major

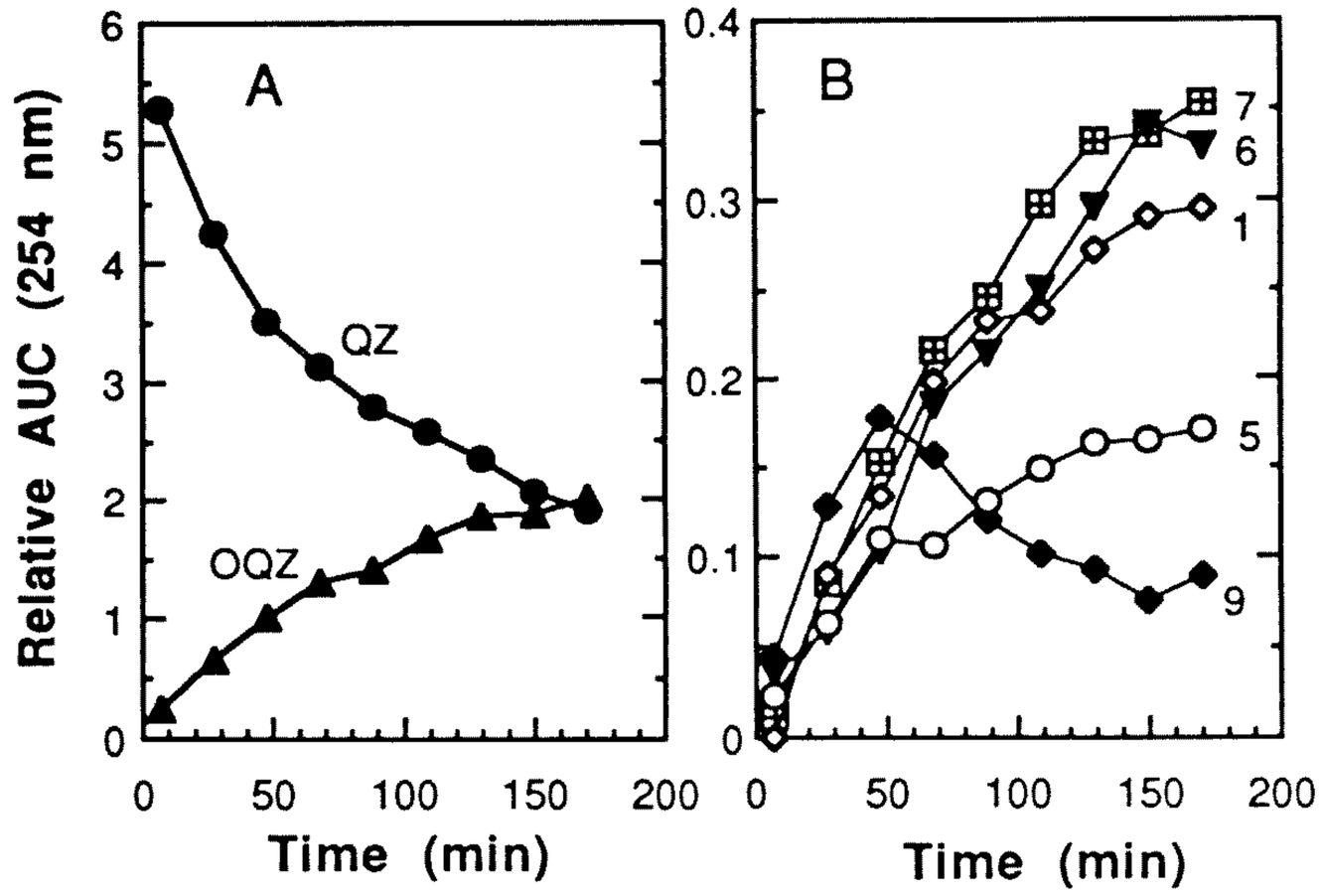


Figure 3. Time course in the formation of hydroxide ion-induced reaction products from QZ. The figure shows : (A) the disappearance of QZ and the formation of OQZ and (B) the formations of peaks 1, 5, 6, 7 and 9 corresponding to those shown in Figure 2. QZ (210 μg) was dissolved in 2 ml of MeCN : 2mM NaOH (1:1,v/v) and maintained at 25°C. Samples were taken at fixed intervals and analyzed by reversed-phase HPLC (see Figure 2 above).

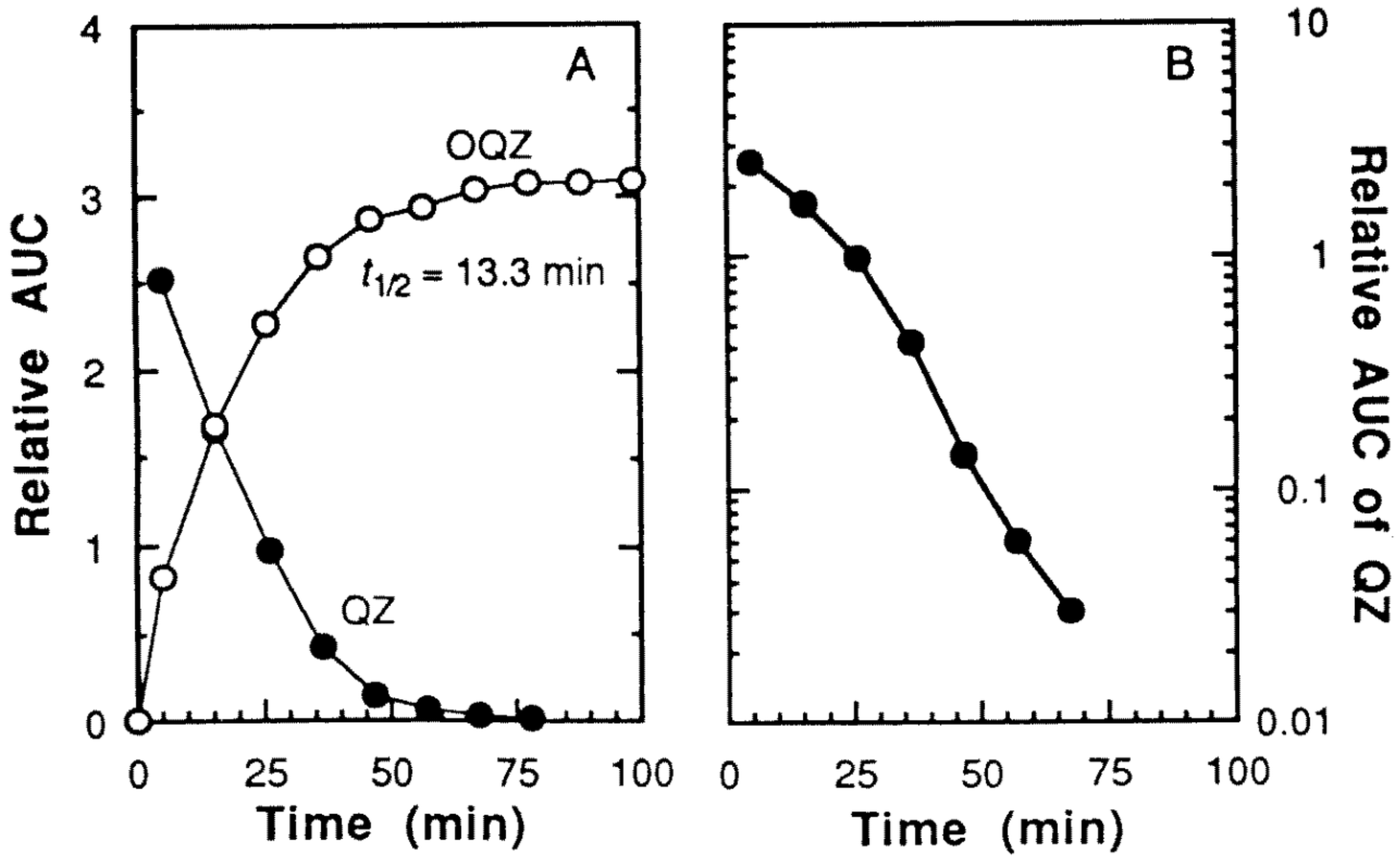


Figure 4. The rates of disappearance of QZ and formation of OQZ. QZ (42 μg) was dissolved in 2 ml of MeCN : 20 mM NaOH (1:1, v/v) and maintained at 25°C. Samples were taken at fixed intervals and analyzed by reversed-phase HPLC.

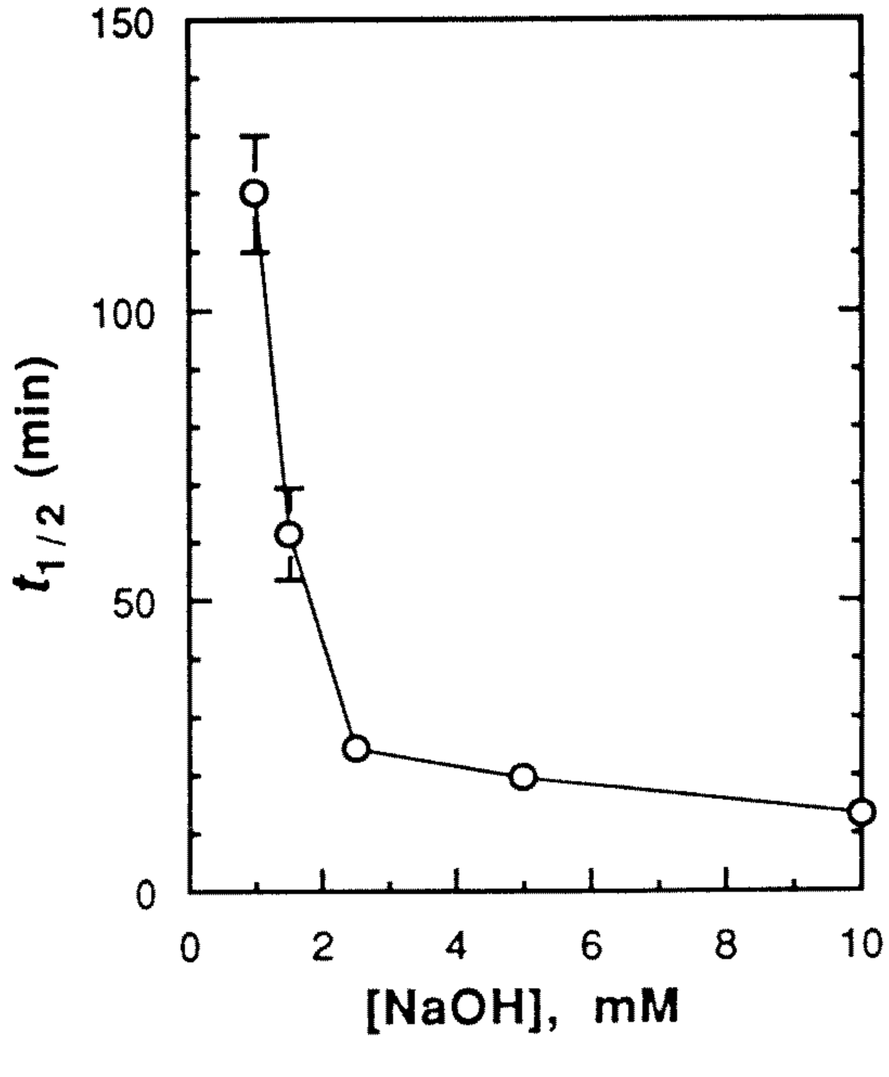
products are shown in Figure 3. It was apparent that the rate of QZ disappearance did not follow simple first-order kinetics. The slower kinetic component in Figure 3A appeared to correspond to the rate of formation of OQZ. The formation of OQZ and those contained in peaks 1, 5, 6, 7 and 8 all increased with time (Figure 3B). The product contained in peak 9 (Figure 2) appeared to be either a reaction intermediate or an unstable product; its amount increased up to 50 min and decreased thereafter.

In order to simplify reaction kinetics, the reaction was studied using 272 μM of QZ and an excess amount of NaOH. In MeCN : 20 mM NaOH (1:1,v/v), the rate in the formation of OQZ ($t_{1/2}$=13.3 min, determined by curve fitting) followed an apparent first-order kinetics (Figure 4A). A semi-log plot in the disappearance of QZ (Figure 4B) indicated a more complex kinetic behavior, probably due to multiple product formations as shown in Figure 2.

The rates in the formation of OQZ were determined as a function of NaOH concentration in 1:1 (v/v) mixtures of MeCN and H_2O (Figure 5). The reaction rate in the formation of OQZ reached a plateau at [NaOH]>2 mM and all reactions followed apparent first-order kinetics.

The results indicated that the reaction was a bimolecular reaction between QZ and NaOH. At [NaOH] : [QZ]>20, the formation of OQZ followed apparent first-order kinetics. Multiple kinetic components were discernible in the rate of disappearance of QZ. These results indicated that the reaction between hydroxide ions and QZ lead to the formation of multiple products and OQZ was the most abundant product formed.

Time-dependent absorption spectra



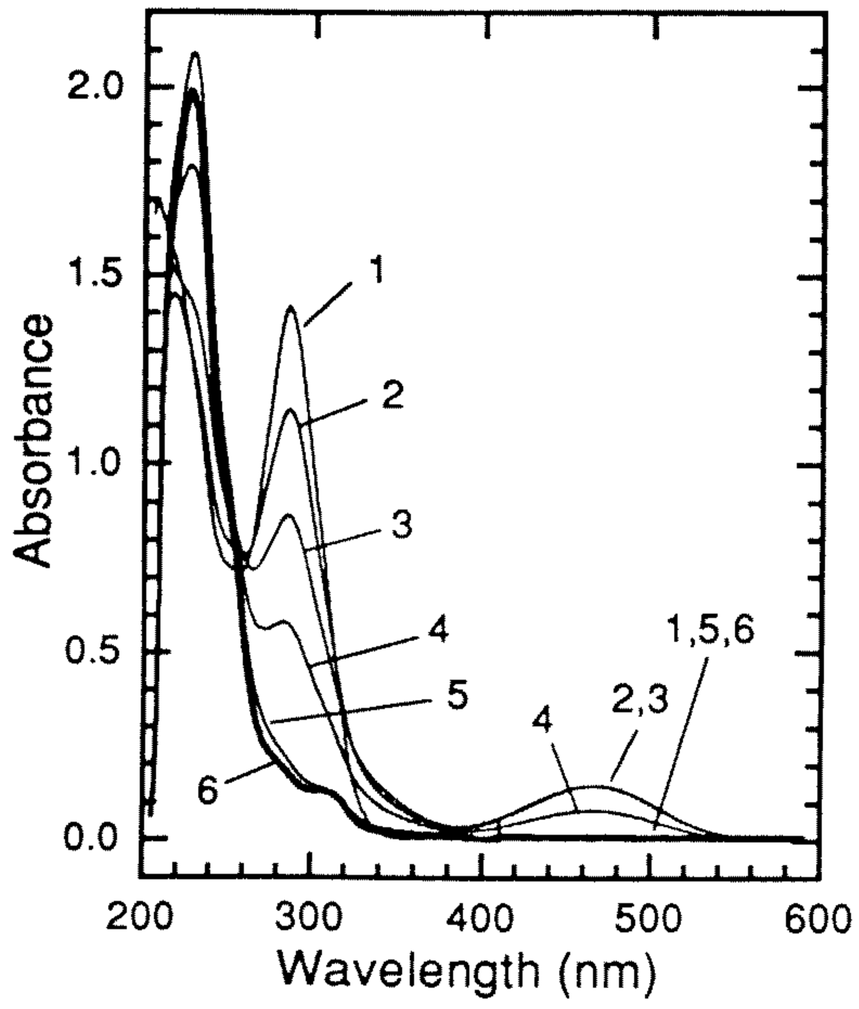


Figure 5. Dependence of reaction t½ in the formation of OQZ on the concentration of NaOH at 25°C. The concentration of QZ was 272 μM. The solvent was MeCN : H_2O (1:1; v/v) containing the indicated concentration of NaOH. Data are mean± SD from triplicate samples.

Figure 6. Time-dependent absorption changes of QZ in an alkaline solution. Spectra 2 to 5 were scans initiated at 0.5, 5, 11, 20 and 70 min following the addition of 2 ml of MeCN : 20 mM NaOH(1:1; v/v) to dissolve 42 μg of dried residue of QZ. Spectra 1 and 6 were QZ and OQZ (in MeCN) respectively. The scan rate was 2 nm/sec.

During studies by reversed-phase HPLC analysis, we noticed the development of an orange-brown color upon addition of NaOH to a MeCN solution of QZ and subsequently the color gradually disappeared. This observation prompted us to monitor changes in uv absorption spectral properties upon addition of NaOH to a MeCN solution of QZ. The results in Figure 6 indicated that, in MeCN : 20 mM NaOH (1:1, v /v) at 25°C, the absorption band of QZ centering at 286 nm decreased at a fast rate in the beginning of the reaction, then, subsequently decreased at a slower rate. At the end of 70 min, the absorption spectrum became essentially that of OQZ. The decrease in absorption band at 286 nm was accompanied by an initial appearance of an absorption band centering at 470 nm. The absorption band at 470 nm completely disappeared in 70 min. It was fonnd that the decrease in absorption at 286 nm coincided with the appearance/disappearance of absorption at 470 nm. These results indicated that our visual observation of color change was due to the formation of intermediate(s) which absorbed at 470 nm.

Reaction kinetics by spectrophotometry

The results in Figure 6 provided two absorption bands centering at 286 and 470 nm respectively, suitable for monitoring the progress of the reaction between QZ and NaOH. Since multiple products were formed from QZ (Figure 2), a complex kinetic behavior was observed in the time-dependent decrease of absorption at 286 nm and 470 nm (Figure 7). The early decrease in A_{286} and increase in A_{470} (Figure 7) indicated the formation of intermediate(s) immediately following the addition of NaOH. In the first few minutes, the increase in A_{470} and the decrease in A_{286} appeared to correspond to the same kinetic process. The formation of OQZ corresponded with the slower kinetic component both in the decrease of both A_{286} and A_{470}(Figure 7) Extensive efforts were made to perform linear regression analysis using various modeling approaches. Unfortunately we could not find kinetic equations that provided a satisfactory fitting to the curves of the experimental data shown in Figure 7. The curves were apparently not due to a consecutive reaction of two first-order kinetics. This was likely due to multiple pathways in the conversion of QZ to various products, as evidenced in the chromatogram shown in Figure 2.

The results in Figures 4 and 7 consistently indicated that an intermediate was formed early in the reaction, which was accompanied by a decrease in A_{286} and an increase in A_{470}. Reaction products were detected as soon as the intermediate(s) was formed, suggesting consecutive reactions. The slower kinetic component coincided with the formation of most abundant product OQZ.

Reaction Mechanism.

Based on the results described above, the mechanism of hydroxide ion-induced conversion of QZ to OQZ is proposed in Figure 8. The reaction is a second-order reaction, first-order in

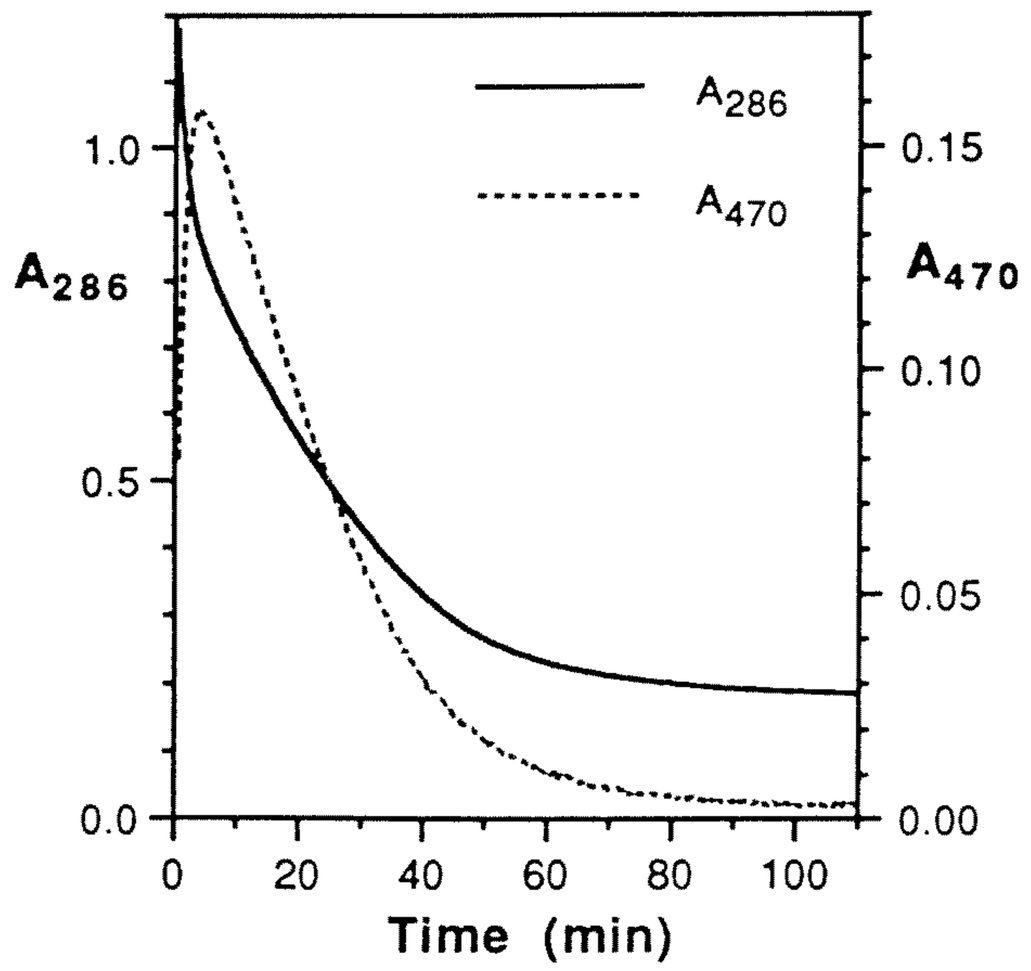


Figure 7. Kinetics of absorption changes at 286 and 470 nm of QZ (54 μM) in MeCN : 20 mM NaOH (1:1; v/v) at 25°C.

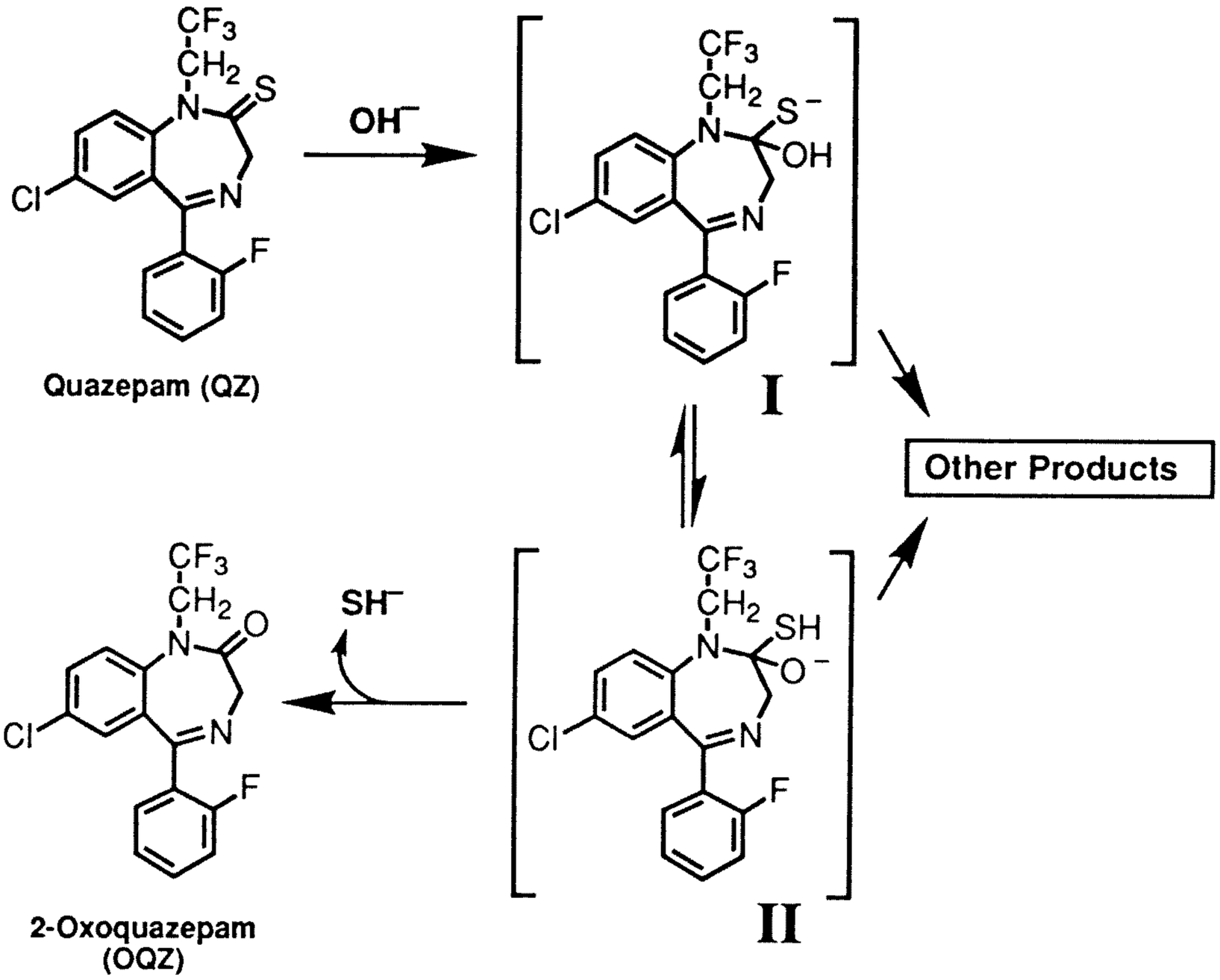


Figure 8. Proposed mechanism in the formation of the major product (OQZ) from QZ in alkaline medium. Structures in brackets indicate proposed intermediates which were not directly isolated and characterized. OQZ may further undergo a basecatalyzed hydrolysis to form OQZ-HP. See text for discussion.

QZ and first order in hydroxide ion. Intermediate I is probably formed resulting from nucleophilic addition of a hydroxide ion to the C2 of QZ. Intermediate I and its isomeric structure II are probably in equilibrium. OQZ is subsequently formed from intermediate II by the loss of SH^-. Intermediate I and its isomeric structure II may be viewed as the transition states of the reaction. OQZ may be further converted to OQZ-HP by base-catalyzed hydrolysis.[13]

CONCLUSIONS

QZ undergoes a second-order reaction in a strongly alkaline media to form predominantly OQZ and some yet unidentified minor products. OQZ is initiated by nucleophilic addition of a hydroxide ion to the electrophilic C2 carbon of QZ, probably due to the electron-withdrawing properties of the 1-N-trifluoroethyl group.

ACKNOWLEDGEMENTS

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the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

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Quazepam在鹼性溶液中轉換成2-Oxoquazepam

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摘　要

Quazepam [7-chloro-1-(N-2,2,2-trifluoroethyl)-5-(2'-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-thione, QZ]是在臨床上使用的一種抗焦慮劑。QZ在鹼性溶液中會轉換成11個以逆相高壓液相層析能檢測得到的產物。其中最多的是2-Oxoquazepam [7-chloro-1-(N-2,2,2-trifluoroethyl)-5-(2'-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, OQZ]。鹼性溶液中的反應動力學經由逆相高壓液相層析法分析，及由紫外與可見光吸收圖譜與時間的依存關係研究指出，QZ轉換成OQZ的反應是先形成一中間體，再形成OQZ主產物。反應機制可能是因QZ的C2缺電子，致使氫氧離子在C2產生親核加成反應，故而形成OQZ及其他之次產物。